

Inhibition of Atrial Anaphylaxis by Corticosteroids^{*1}

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Anaphylactic reaction in the heart isolated from sensitized guinea pigs has been studied extensively by NAKAZAWA and his collaborators using isolated atria, whole hearts or ventricle strips. The anaphylactic reaction in those preparations are found generally as an augmentation of contractile force and acceleration of rate. In the present paper, an attempt was made to evaluate the inhibiting effect of corticosteroids and their analogs to the anaphylactic reaction in isolated atria because the reaction was considered semiquantitative. As the results, corticosteroids administered in vitro showed no preventive effect on the anaphylactic reaction of the atria. Glucocorticoids administered in the sensitized animal prior to preparation showed inhibition of augmentation of contractile force in the anaphylactic reaction, but no inhibition was observed in its accelerating effect. Desoxycorticosterone given in vivo was found to increase the amplitude enhancing effect in atria anaphylaxis and also to enhance, histamine, serotonin and adrenaline action.

There is a big difference between heart anaphylaxis and other anaphylactic reactions occurred in different organs such as Schultz-Dale's reaction using intestinal tracts or uterine segments. The former shows more dynamic responses, occurring in different muscle tissue having different innervation. Moreover, physiologic states of the heart before the anaphylactic reaction can easily be known by its natural rhythm and its contractions.

NAKAZAWA⁽⁶⁾⁽⁷⁾⁽¹⁰⁾ and his co-workers have studied extensively about heart anaphylaxis, namely an anaphylactic reaction which appears when the antigen is added to isolated atrium or heart taken from previously sensitized animals. KIHARA⁽⁶⁾⁽⁷⁾ reported on participation of various antihistaminic agents to the atrial anaphylaxis, concluding that the agents could not be antianaphylactic. And also criticized the significance of acetylcholine as a basic active substance most responsible to anaphylactic manifestations, and so on.

Since the advance of corticosteroids, many attempts have been made to apply the hormone and its artificial analogs to various allergic

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states. And some of them have afforded satisfactory symptomatic relief. But there are a few observations concerning the antianaphylactic effect of active steroids on experimental anaphylaxis in the guinea pig. On the other hand, the hormone have progressed greatly in its biological activities by changing in chemical configuration of the steroid nucleus and its side chains.

In the present paper, an attempt was made to observe the effect of active steroids on experimental anaphylaxis using isolated atrium of the guinea pig which was previously sensitized to egg albumin. The method provides that the physiologic state of the organ before anaphylactic examination can be selected uniformly, and the results seem to be considered as semiquantitative enough.

METHODS AND MATERIALS

Male and female guinea pigs weighing between 250 to 300 g kept in the laboratory diet for 2 weeks at least were used for the experiment. Anaphylactic sensitization of the guinea pig has carried out by injection of 0.3ml/100g body weight of anti-egg-albumin rabbit sera whose antibody titer were between 1×64 to 1×128 . The injection was made 18 to 24 hours prior to evocation of anaphylactic shock.

The atria were dissected from freshly killed guinea pigs and suspended in Lock's solution at 29°C. The solution was saturated with oxygen containing 5% carbon dioxide. The contractions of the atrium were recorded on a smoked drum by an isotonic lever which responds linearly up to 200 beats per minutes. Isometric records of contractions were carried out by means of Shinkoh U-Gauge (unbonded strain gauge) in a part of the experiment. Every experiment was started after the contractions and rhythm of the atria became constant. It usually needed 40 to 60 minutes after suspension of the atrium into bath. Solution of egg albumin as the antigen was added into bath at a rate of 0.02% of Lock's solution every time. Before adding the antigen into the bath antifoaming treatments using such as Silicone or cetyl alcohol were essentially needed because of the solution of egg albumin having great foaming quality. The changes in both contractile amplitude and rate of atrial movements were expressed as percentage to those of just prior to addition of antigen or drugs.

Corticosteroids and their analogs used in this experiment are as follows; sodium 17-hydroxycorticosterone-21-succinate (Solu-Cortef; Upjohn), desoxycorticosterone glucoside (Cortiron intravenous; Schering), 1, 6, 2-methyl-9-fluoro-prednisolone-21-phosphate disodium (Dexamethasone; Merck).

RESULTS

1. Pattern of normal atrial anaphylaxis: An amplitude enhancing (augmentation of contractile force) and rhythm accelerating effects were observed 30 to 40 seconds after addition of antigen into bath (Fig. 1).

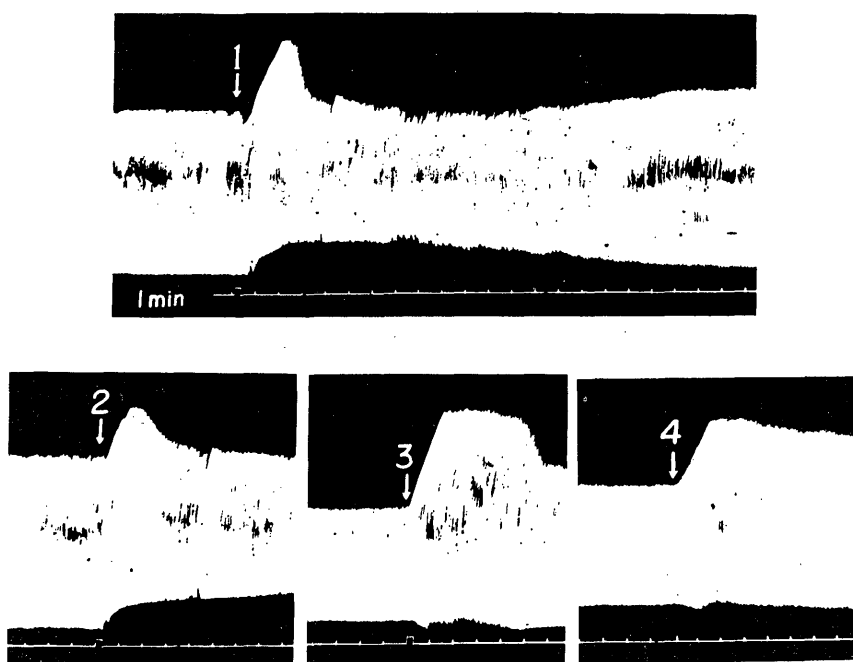


Fig. 1. Anaphylactic reaction and response to drugs of an atrium isolated from a sensitized guinea pig.

1 : Antigen (egg albumin 0.2mg/ml) 2 : Histamine 0.2 μ g/ml
3 : Adrenaline 0.01 μ g/ml 4 : Serotonin 2.0 g/ml

Average values of maximal responses in amplitude and beat was 30% up respectively. These effects were continued for some 15 minutes, and returned to its normal activities. Such accelerating effects of the atria were never observed when the antigen was added to isolated atria from non-sensitized guinea pigs. So the accelerating effects should be an anaphylactic reaction occurred only in the atria from sensitized animals.

2. Similarity of atria anaphylaxis with effect of some agents: An appropriate amount of histamine, adrenaline or serotonin caused accelerating effects similar to anaphylactic reaction on the isolated atria (Fig. 1). Effect of adrenaline (0.01 μ g/ml) was terminated after 10 minutes. Effects of serotonin (2.0 μ g/ml) and histamine (0.2 μ g/ml) continued over a period of 60 minutes. But acetylcholine caused only inhibiting effect to the atria. This has been studied extensively by KIHARA⁷⁾ previously.

Table 1.
Changes in Amplitude Enhancing Effects of Anaphylaxis and
other Drugs by Premedication of Corticosteroids

	Mean† of % Increase in Contractile Amplitude			
	Untreated	HC	DM	DOCG
Egg Albumin 0.2mg/ml	30±3.3	6.8**±1.9	3.6**±1.6	68**±4.8
Histamine 0.2μg/ml	36±4.9	35±5.2	39±3.8	73**±4.5
Serotonin 2μg/ml	39±5.0	35±2.0	32±3.1	64* ±9.5
Adrenaline 0.01μg/ml	52±6.8	51±5.2	50±4.5	82**±4.8

† Each value represents mean of at least 4 experiments ± standard error.

** Statistically significant at 1% level.

* Statistically significant at 5% level.

HC, hydrocortisone 4mg/100g body weight; DM, dexamethasone 0.2mg/100g; DOCG, desoxycorticosterone 2mg/100g.

3. Influence of steroids on atrial anaphylaxis:

a) Administration of corticosteroids *in vitro*.

hydrocortisone Sodium 17-hydroxycorticosterone-21-succinate was administered into the bath (100μg/ml) suspending isolated atria from the sensitized guinea pig. The beat was gradually slowed by the treatment. And 60 minutes later, it became 20% down to the rate before addition of hydrocortisone. Under this condition, anaphylactic reaction was also evoked by addition of the antigen similar to that in the atrium not treated with the steroid (Fig. 2). The atria responded normally to histamine.

dexamethasone Dexamethasone-21-phosphate disodium given *in vitro*

Table 2.
Changes in Positive Chronotropic Effects of Anaphylaxis
and other Drugs by Premedication of Corticosteroids

	Mean ⁺ of % Increase in Heart Rate			
	Untreated	HC	DM	DOCG
Egg Albumin 0.2mg/ml	30±3.0	24±3.4	29±0.7	38±3.9
Histamine 0.2μg/ml	23±4.6	27±2.3	22±1.2	30±4.4
Serotonin 2μg/ml	11±2.3	14±1.1	16±3.1	20±3.5
Adrenaline 0.01g/ml	26±3.0	26±2.2	25±2.3	26±2.5

⁺ Each value is the mean of at least 4 experiments ± standard error

HC, hydrocortisone 4mg/100g; DM, dexamethasone 0.2mg/100g;

DOCG, desoxycorticosterone 2mg/100g.

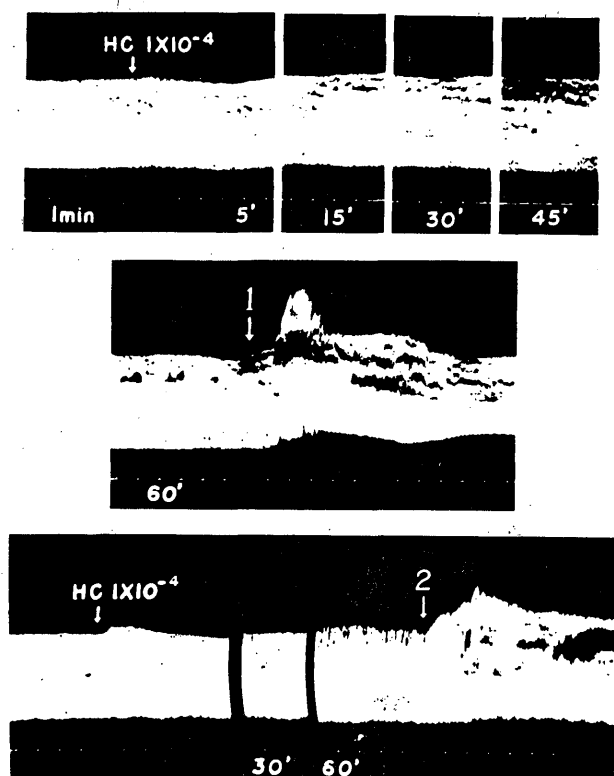


Fig. 2. Anaphylaxis and effect of histamine after in vitro administration of hydrocortisone.

HC: Hydrocortisone sodium succinate. 1 : Antigen

2 : Histamine 0.2 μ g/ml.

(20 μ g/ml) gradually diminished the contractile amplitude of the isolated atria. The antigen was added into the bath about 60 minutes after the administration of dexamethasone. And the same anaphylactic reaction was observed as in the untreated case.

desoxycorticosterone. Desoxycorticosterone glucoside (10 μ g/ml) caused a negative chronotropic effect on the isolated atria. But the anaphylactic reaction was not influenced by the treatment. And the preparation responded normally to histamine.

b) Administration of corticosteroids *in vitro*

hydrocortisone Sodium 17-hydroxycorticosterone-21-succinate was injected intravenously (4mg/100g body weight) to sensitized guinea pigs 24 hours before sacrificing animals. The drug of this dose caused no changes in appearance and activities of the guinea pig. The isolated atria from thus treated animals showed also no change in its movement, and anaphylactic reaction was evoked normally.

While the same dose of hydrocortisone was injected 1 to 2 hours prior to the experiment, it is noted that the amplitude enhancing effect

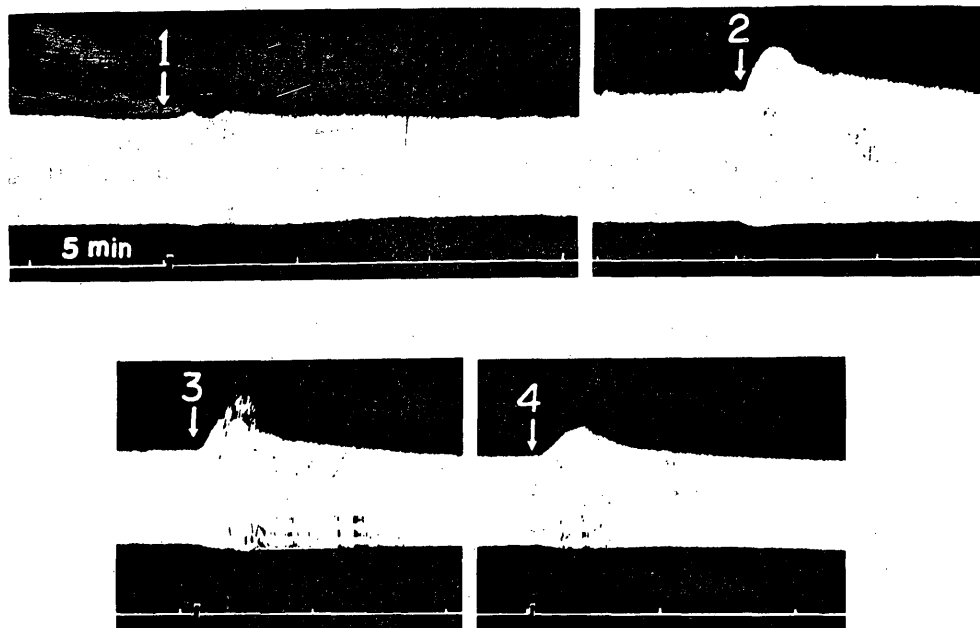


Fig. 3. Anaphylaxis and response to drugs of the atrium isolated from a hydrocortisone administered guinea pig.

1 : Antigen (egg albumin 0.2mg/ml) 2 : Histamine 0.2 g/ml
3 : Adrenaline 0.01 g/ml 4 : Serotonin 2.0 g/ml

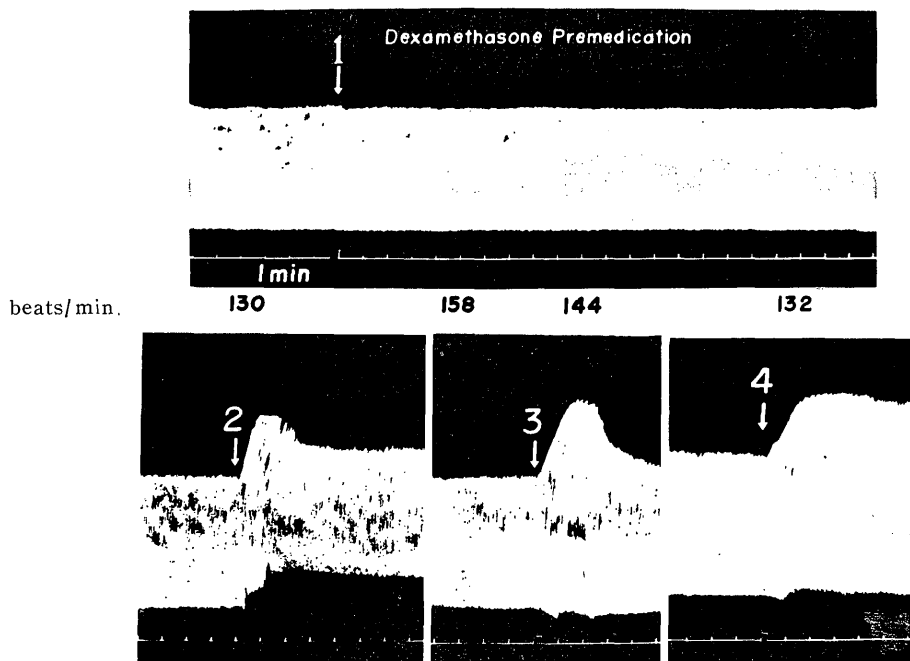


Fig. 4. Anaphylaxis and response to drugs of the atrium isolated from a dexamethasone pretreated guinea pig.

1 : Antigen (egg albumin 0.2mg/ml) 2 : Histamine 0.2 μg/ml
3 : Adrenaline 0.01 μg/ml 4 : Serotonin 2.0 μg/ml

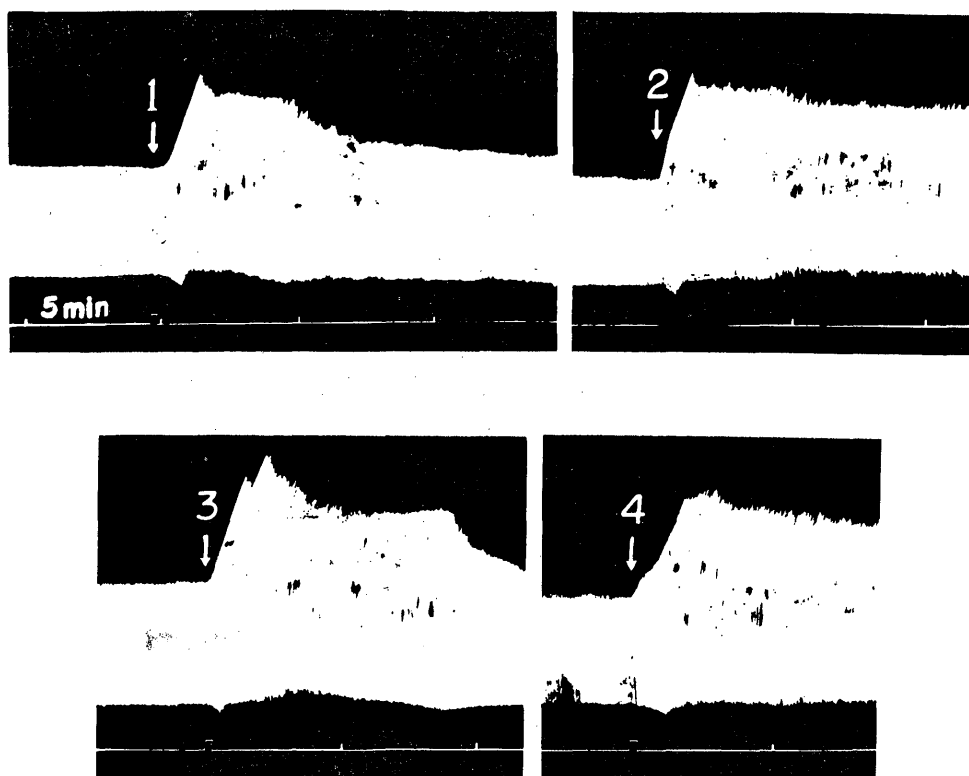


Fig. 5. Anaphylaxis and response to drugs of the atrium isolated from a desoxycorticosterone pretreated guinea pig.

1 : Antigen (egg albumin 0.2mg/ml) 2 : Histamine 0.2μg/ml
3 : Adrenaline 0.01μg/ml 4 : Serotonin 2.0μg/ml

of anaphylaxis was markedly inhibited (Fig.3). But no significant inhibition was observed in the rhythm accelerating effect of the anaphylaxis. The atria responded normally to histamine, serotonin and adrenaline (Fig. 3) The result listed in Table 1 and 2 gives average values from four experiments at least.

dexamethasone An amplitude enhancing effect of the anaphylactic reaction was completely or markedly inhibited by administration of dexamethasone (0.2mg/100g) 1 to 2 hours before sacrificing of animals (Fig. 4). But the treatment had no effect on its rhythm accelerating effect as shown in Table 2. The effect of histamine, serotonin and adrenaline on atrial movement was observed normally.

desoxycorticosterone Desoxycorticosterone glucoside was administered intravenously (2mg/100g) to sensitized guinea pigs 60 minutes prior to sacrifice. The atria responded more markedly to addition of the antigen than in the case of untreated guinea pig (Fig. 5). As shown in Table 1, contractile responses of the antigen, histamine, serotonin and adrenaline were augmented by premedication with desoxycorticosterone, although no significant change in the beat was observed.

DISCUSSION

It is well known that the course of anaphylactic phenomenon occurred in whole animals is so complicated. So the attempt of the present experiment was made to observe the effect of corticosteroids and their analogs on anaphylactic reaction in the isolated atria.

Participation of corticosteroids on antibody formation was reported by many investigators. CHASE *et al*³⁾ reported that adrenocortical extracts increased antibody formation in mice, rats and rabbits. DEWS and CODE⁴⁾ reported that antibody formation in guinea pigs was not influenced by cortisone and ACTH. ARBESMAN *et al*¹⁾ have reported that reversed or Forssman anaphylaxis was inhibited by ACTH and cortisone through no influence on antibody formation. In the present experiment, a passive sensitization with anti-egg-albumin rabbit sera was used for the experiment. So the effect of steroids on anaphylaxis through its participation to antibody formation can be neglected from the discussion. Role of inhibition of corticosteroids upon anaphylaxis should be searched for other than antibody formation.

It has been reported by DEWS and CODE⁴⁾, NELSON *et al*¹¹⁾, and MIKULICICH and OESTER⁹⁾ that anaphylactic reaction in rats, mice and rabbits was prevented by corticosteroid. But general anaphylactic reactions in the guinea pig were not inhibited by corticosteroids^{1,5,8)}. In most experiments, however, the result can be obtained satisfactorily through interpretation of the reaction of all or none, because it usually have great individual differences.

The anaphylactic reaction in the atria prepared from sensitized guinea pigs treated with hydrocortisone or dexamethasone 1 to 2 hours prior to sacrifice was partly inhibited, while it was not influenced at all when the animals were treated before 24 hours. On the other hand the atria responded normally to histamine, serotonin and adrenaline. Hydrocortisone or dexamethasone treatment *in vitro* had no influence on the anaphylactic reaction and on the response of histamine. BASS and SETLIFF²⁾ have pointed out a spasmolytic action of various active steroids against the contraction with acetylcholine or barium chloride in intestinal and uterine segments. In the atria, however, it was observed that the responses to histamine, serotonin and adrenaline were not influenced by treatments with corticosteroids both *in vitro* and *in vivo*, with an exception of desoxycorticosterone. A difference in the effect of steroids to anaphylaxis found between administrations *in vitro* and *in vivo* suggests that the effect on anaphylaxis are due to mechanism other than spasmolytic action and that the specific inhibition of the anaphylaxis appeared as a secondary effect through a modified metabolic process by the steroids.

SUMMARY

Effects of corticosteroids on the anaphylactic manifestation in isolated atria prepared from passively sensitized guinea pigs were investigated.

1) Hydrocortisone, dexamethasone and desoxycorticosterone added *in vitro* showed no inhibitory effect to the anaphylaxis and to the effect of histamine.

2) An inhibitory effect was found in anaphylactic reaction of the atria when hydrocortisone or dexamethasone were administered intravenously 1 to 2 hours prior to sacrifice.

3) Anaphylactic reaction and the response to histamine, serotonin and adrenaline were augmented by *in vivo* administration of desoxycorticosterone.

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